Genetic Variation of *In Vitro* Rooting Ability With Time in *Eucalyptus globulus*

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(Received 18th August 1998)

Abstract

The genetic control of *in vitro* rooting success of *Eucalyptus globulus* was examined in two studies, one involving 496 ortets (seedlings) from 20 open pollinated families from a single race (Jeeralang) and another involving 155 ortets from 15 control pollinated families (half-diallel). Rooting was examined in several consecutive rooting tests, spanning a one year period, allowing the importance of genetic effects, changes over time, and the interaction between genotype and rooting tests to be determined. Significant levels of additive genetic variation for rooting success were detected. The narrow-sense heritability was h^2=0.16 and 0.27, respectively with the genetic correlations across tests extremely high (0.83 and 0.81). The levels of specific combining ability and ortet (within family) by test interaction were low (1.5% and 5% of the total variation, respectively) but significant. Rooting ability dropped markedly during the initial few months but seemed to remain stable thereafter, at levels of 15% and 40%, respectively.

Parents with an intrinsic genetic superiority for rooting can be identified on the basis of a few well replicated tests. The superiority is expected to be mostly under additive genetic control which would allow the use of bulked family deployment strategies as well as breeding for improved rooting ability.

Key words: in vitro rooting cloning, *Eucalyptus globulus,* genetic variation, heritability.

FDC: 165.3; 165.44; 181.36; 232.328.1; 176.1 *Eucalyptus globulus.*

Introduction

Increased genetic gains through clonal deployment of superior genets or families is well recognised in eucalypts (e.g. ZOBEL, 1993; BARBOUR and BUTCHER, 1995; MACRAE and COTTERILL, 1997). This is a common strategy for deployment of tropical or sub-tropical eucalypt species such as *E. grandis, E. urophylla* and *E. camaldulensis* which are readily clonally propagated through hardwood cuttings or *in vitro* techniques (HARTNEY, 1991; LEROUX and VAN STADEN, 1991). By contrast, poor cloning ability of the major temperate species *Eucalyptus globulus* ssp. *globulus* and *E. nitens* (WILLYAMS *et al.,* 1992; WILSON, 1992; BARBOUR and BUTCHER, 1995; TIBBITS *et al.,* 1997) has effectively restricted deployment to seed orchards (BORRALHO, 1997) or, more recently, mass controlled-pollination (LEAL and COTTERILL, 1997). Where clones of these species have been deployed, this is usually following extensive screening for vegetative propagation ability (WILSON, 1992). However, numerous selections must be discarded because of failure to reach economically viable levels of rooting (greater than 70%; WILSON, 1992). This will reduce the number of genets available for deployment and the potential gain in traits of direct economic significance (MACRAE and COTTERILL, 1997).

Many years of research have failed to yield technological solutions to substantially decrease the level of recalcitrant genets in these temperate species for either micropropagation or hardwood cuttings (MACRAE and COTTERILL, 1997). An alternative solution is to directly select for propagation characteristics (HAINES and WOOLASTON, 1991) in the main or elite breeding populations and improve the cloning potential of these populations. Genetic gains with this approach will, however, depend upon the variation and heritability of propagation characteristics such as survival and rooting potential. There is considerable phenotypic variation between genets of these species for their ability to produce roots in either micropropagation (WILLYAMS *et al.,* 1992) or stem cutting (DE LITTLE and RAVENWOOD, 1991; WILSON, 1992, 1993) systems, with mean percentage rooting ranging between 0% and 100% for hardwood stem (WILSON, 1992) and *in vitro* (WILLYAMS *et al.,* 1992) cuttings. This variation has been shown to be moderate to highly heritable for stem cuttings of *E. globulus* ssp. *globulus* (BORRALHO and WILSON, 1994; ENGLAND and BORRALHO, 1995; LEMOS *et al.,* 1998) and *E. nitens* (TIBBITS *et al.,* 1997). However, there is little published information on the genetic basis of variation in propagation ability in micropropagation systems and how this changes with time, despite the generally held view that tissue culture techniques may be able to overcome some propagation constraints. Genetic differences at the family and provenance (Jeeralang vs Otways vs Moogara) level have been reported in *E. globulus* ssp. *globulus* by WILLYAMS *et al.* (1992) and the genetic control of micropropagation characteristics of *E. nitens* x *globulus* hybrids has been reported by RASMUSSEN *et al.* (1995). However, there are no published reports of the heritability and levels of non-additive genetic variation for such characteristics in *E. globulus.*

The present paper examines the level of genetic control of rooting ability from micropropagation, across a number of consecutive rooting tests. Two experiments are presented, the first using open pollinated families of *Eucalyptus globulus* from a single race (Jeeralangs; considered an intergrade between ssp. *globulus* and either *bicostata* or *pseudoglobulus*), and the second using control pollinated families from a half diallel between *E. globulus* ssp. *globulus* parents. The study investigates the variation in rooting ability with time, and the importance of additive and non-additive genetic effects and how these effects change across tests.

Material and Methods

Genetic material

Experiment 1

The ortets (496 in total) were derived from 20 open-pollinated families, with seed collected in a native stand from the Jeeralang provenance (see DUTKOWSKI and POTTS, 1999). Each family was represented by approximately 25 ortets.

Experiment 2

The 155 ortets in the experiment were crosses between 6 fully pedigreed parents, mated in a incomplete half-diallel

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design such that each family had unrelated parents *(Figure 1)*. Two of the families were reciprocal crosses. Of the 11 grandparents, six were of unknown origin (4, 10, 11, 20, 23), whereas the other five originated from different provenances of *E. globulus* in south-eastern Tasmania (3 = Tinderbox; 5 and 9 = Hobart; 28 = Taranna; 29 = Bruny Island). There was an average of 10 ortets per family, but ranging from 1 to 15.

	Male					
Female	**11 x 5**	**4 x 3**	**14 x 9**	**23 x 20**	**23 x 10**	**28 x 29**
11 x 5						
4 x 3	4					
14 x 9	14	2				
23 x 20	14	10	12			
23 x 10	12	7	11			11
28 x 29	14	1	15	14	14	

Figure 1. – Half-diallel mating design used in experiment 2. The pedigree of each parent is indicated and the number of individuals in each cross are shown.

Micropropagation techniques

Seeds were disinfected with a solution of commercial bleach (4% active chlorine) for 8 minutes, rinsed three times in sterile deionised water, and then sown singly into autoclaved 500 ml containers containing 100 ml of washed perlite and 50 ml liquid medium (1/4 MURASHIGE and SKOOG (1962) mineral medium plus 1.5% sucrose).

Containers were placed in a controlled environment: 21°C / 16°C ± 1°C thermoperiod associated with 16 h / 8 h photoperiod provided by Sylvania Day Light fluorescent tubes supplying 70 to 80 µmol m^2s^{-1}. Every 6 weeks the liquid phase was removed and replaced with fresh liquid medium. The seedlings (ortets) were pruned to develop multiple branches and cuttings used for rooting tests. For testing, these cuttings were cut to three nodes plus the apex, the basal pair of leaves removed and the intermediate pair cut transversally, removing half of the leaf. Seedlings were first pruned after 9 (Expt. 1) and 12 weeks of culture, and then approximately every 6 weeks at the time of changing the medium. The original seedling was always used as the source of new shoots. Cuttings harvested during the first year in experiment 2 were used to define a rooting medium. As a good compromise of root-induction efficiency and quality of rooting (no or very little callus formation) the testing medium used consisted of the same basal medium for growing the plantlets plus 1 µM Indolebutyric acid (IBA) and 5 $g.l^{-1}$ agar. Cuttings were laid on solid rooting medium (25 ml in Petri dishes 90 mm diam.) with the cut section in the gelled medium. These cultures were placed under low light intensity, 15 µmol $m^{-2}s^{-1}$ for 3 weeks. For each test, cuttings from the same ortet were placed in the same Petri dish and the position of the Petri dishes was randomised regularly during the test period. Rooted cuttings were counted 3 weeks after setting, when cuttings with roots could be easily distinguished.

The rooting tests in experiment 1 were conducted in March 1996, May 1996, June 1996, July 1996, September 1996 and February 1997. Each test consisted of an average of 3 ramets per ortet, but ranging between 1 and 5. The tests in experiment 2 were undertaken between February and June 1996, with each having between one and three ramets per ortet. The number of replicates per ortet per test is limited by the regeneration potential of each plant and is thus necessarily low. However, this is not a limitation as we are concerned with estimating levels of genetic variation and hence variance components rather than trying to accurately predict the rooting of any one ortet or family.

Statistical analysis

The estimation of variance components utilised individual tree, restricted maximum likelihood (REML) mixed models following BORRALHO and WILSON (1994) and LEMOS *et al.* (1998). The individual tree approach is ideally suited to the analysis of imbalanced, multi-generation pedigrees and uses know pedigree information to constructed an additive relationship matrix from which additive effects are estimated directly (GILMOUR *et al.*, 1995; LEMOS *et al.*, 1998). The analysis of the data for experiment 1 followed the model:

$$y = t + a + p.t + o.t + e \qquad [1]$$

where y is the vector of observed rooting ability per ortet in each test, given as the percentage rooting out of n ramets, t are the test effects (fixed), a is the additive genetic effect, $p.t$ and $o.t$ are the effects due to female parent by test and ortet (within family) by test interactions respectively, and e is the residual. An iterative weighted Restricted Maximum Likelihood analysis (COLLET, 1991) was used to obtain the weights, as $w_{ij} = [p_{ij}(1-p_{ij})/n_{ij}]^{-1}$, where p_{ij} is the predicted proportion and n_{ij} number of ramets for the i^{th} ortet in the j^{th} test. Heritability estimates assumed a coefficient of relatedness between open pollinated sibs of 0.25. Genetic correlations between tests were approximated by:

$$r_G = \frac{V_A}{V_A + V_{P.T}} \qquad [2]$$

Analysis of the data for experiment 2 followed the model:

$$y = t + a + p.t + o.t + sca + s.t + e \qquad [3]$$

where y is the vector of observed rooting ability per ortet for each individual ramet, hence given as zero or one, t is the test effects (fixed), a is the additive genetic effect, $p.t$ are the effects due to parent by test interaction, $o.t$ are the effects due to ortet (within family) by test interactions, *sca* is the effect due to specific combining ability, $s.t$ are the effects due to sca by test interaction and e is the residual.

Both analysis assumed a binomial distribution of the data, and a logit link function. Variance components and associated standard errors were estimated using the program ASREML (GILMOUR *et al.*, 1995). The statistical significance of the variance components was tested using the log likelihood ratio test (SHAW, 1991).

Individual narrow-sense heritabilities were calculated in experiment 1 as:

$$h^2 = \frac{V_A}{V_A + V_{P.T} + V_{O.T} + V_e} \qquad [4]$$

and for experiment 2 as:

$$h^2 = \frac{V_A}{V_A + V_{P.T} + V_{O.T} + V_{SCA} + V_{S.T} + V_e} \qquad [5]$$

Results

Mean rooting ability

Overall mean rooting percentage across the six tests was 36% and 38%, for experiments 1 and 2. However, in experiment 1, rooting success decreased significantly with time, from a high rooting of 67% in the first test, only 60 days after germination, to around 15% from 100 days onwards *(Fig 1)*. In experiment 2, there was also a significant drop in rooting, from the first (with 35%) to the third test (with 23%) *(Figure 2)*.

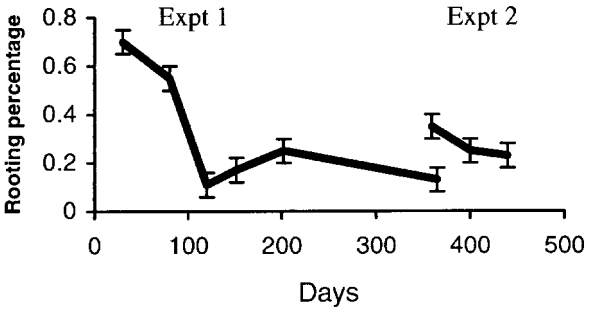

Figure 2. – Generalized Least Square Means for rooting percentage in the two experiments. The horizontal axis represents number of days since the *in vitro* germination of the seed. Vertical bars refer to ± one standard error.

Distribution of good rooters

The very best ortets were up to 2 standard deviations above the mean (one standard deviation being around 40%), with outstanding rooters coming mostly from a single family. For example, of the best 25 ortets in experiment 1 (i.e. the top 5%), 15 came from one family alone, with the remaining 10 coming from 7 other families.

Variance components

Significant (P<0.001) additive genetic variation for rooting ability was detected in both experiments, with across test heritability of $h^2=0.16$ and 0.27 for experiments 1 and 2, respectively. In experiment 1, a half-sib structure was assumed, hence the estimate should be seen as an upper limit. A coefficient of relationship of 1/2.5, instead of $^1/_4$, is often used in open pollinated studies in eucalypts, to adjust for the occurrence of selfed and outcrossed full-sibs in the open pollinated progeny (POTTS *et al.,* 1995). This would reduce further the heritability in experiment 1 to $h^2=0.10$.

In experiment 2, the specific combining effect (V_{SCA}) was small and not statistically significant, accounting for 1.5% of the total variance. There was also no significant SCA by test interaction ($V_{S.T}$). The ortet (within family) by test interaction ($V_{O.T}$) was significant in both experiments but also relatively small, accounting for 4% and 5% of the total variance, respectively. Parent by test interaction (V_{PT}), on the other hand, was close to zero and not statistically significant. In other words, parent and family rankings were stable as the experiment progressed. Ortets within families appeared somewhat more interactive, but this could be due to Petri-dish effects rather than true genotype x environment interactions. As a result, genetic correlations between tests within an experiment were high, with estimates of 0.83 ± 0.11 and 0.81 ± 0.11 for experiment 1 and 2, respectively.

Discussion

Rooting success with this micropropagation technique was initially very high, as is often found in short term tissue culture experiments (LEROUX and VAN STADEN, 1991), but dropped quickly with time to levels similar to that found in long term cuttings experiments (WILSON, 1993; LEMOS *et al.,* 1998). In experiment 1, a high rooting ability of around 60% was observed in the first two tests, only a few weeks after germination, but it quickly declined to around 15%. In experiment 2, there was also a significant drop between the first test (with 35%) and the third test (with 23%). Maturation may be involved in these reductions, at least in experiment 1. Alternatively, the propagation strategy adopted, of always going back to the original seedling as a source of new shoots, may also play a role and deteriorating quality of the growing environment of the ortet can not be discounted. Repeated harvests can reduce the vigor and therefore the quality and rooting competence of the shoots. In experiment 1 new shoots were taken every six weeks from the same original seedling. Rooting ability did not drop as low as in experiment 2 despite the long period of maturation of the seedlings which were growing *in vitro* for 12 months, suggesting that reduced vigor from frequent harvests, rather than maturation, could also be a reason for the observed decline in experiment 1. Overall, these results suggest long term rooting success of tissue culture propagation should range between 15% and 25%. The best ortets, however, performed consistently well, being one to two standard deviations better across the whole experiment (i.e. around 80% across the whole experiment). Despite a relatively large within family variation, at least in one of the experiments, the best rooters are mostly coming from a single family.

A surprising result was the low heritability found in the two studies, at least compared with previous estimates using cuttings (BORRALHO and WILSON, 1994; ENGLAND and BORRALHO, 1995; LEMOS *et al.,* 1998). This was found despite the *in vitro* conditions, and the relatively stable performance of families across tests. Micropropogation experiments are not free of experimental errors, due for example to contamination, handling and poor plant vigor. Although these can be improved with time and experience, the results suggest that rooting ability remains a problematic trait to control and assess even using *in vitro* techniques. Given the long time span of both experiments, the low genotype-by-test interactions suggests the clonal merit for rooting should be accurately estimated from a few well established rooting tests early in the propagation phase. Low genotype-by-test interaction has also been reported by BORRALHO and WILSON (1994) and LEMOS *et al.* (1998) using cuttings. Nevertheless, the low heritability in experiment 1 may also be due to the relatively narrow genetic basis, which only include families from the Jeeralang race, whereas the grandparents in experiment 2 were from several races.

Table 1. – Variance components for additive genetic (V_A), specific combining effects (V_{SCA}), parent by test interaction (V_{PT}), ortet (within family) by test interaction (V_{OT}), SCA by test interaction (V_{ST}), residual effects (V_E), and heritability with its standard error, in the two experiments. Estimates assume an underlying binomial distribution.

Expt	V_A	V_{SCA}	V_{OT}	V_{ST}	V_{PT}	V_E	h^2
1	0.640	–	0.151	–	0.003	3.29	0.16± 0.02
	***		***		ns		
2	1.403	0.077	0.258	0.022	0.074	3.29	0.27± 0.06
	***	ns	***	ns	ns		

*** = significant at 0.1% level, ns = not significant at 5% level

Despite the reduced number of parents in the diallel, in experiment 2, specific combining effects seem small compared with additive effects. Low specific combining effects were also found in large hardwood cutting experiments of *E. globulus* (LEMOS *et al.,* 1998) and *E. nitens* (TIBBITS *et al.,* 1997). The low specific combining effects ensures that the performance of an untested cross should fall close to the mean genetic merit of both parents. Providing selection accuracy of the parents is good, crosses between outstanding rooters should therefore produce families of outstanding rooting ability for deployment. The statistically significant levels of additive genetic variation detected within *(Table 1)* and between (WYLLIAMS *et al.,* 1992) populations indicates that there is potential for improving rooting ability in *Eucalyptus globulus.* Genetic gains could be rapidly made for this trait by crossing high rooting genets already growing in clonal trials or clonal plantations. However, the low heritability of this trait would strongly argue for the utilisation of information on the performance of relatives (through index or BLUP selection) to increase the accuracy of individual selection.

References

BARBOUR, E. L. and BUTCHER, T.: Field testing vegetative propagation techniques of *Eucalyptus globulus.* In: POTTS, B. M. *et al.* (Eds.): Eucalypt Plantations: Improving Fibre Yield and Quality. pp. 313-314. Proc. CRC-IUFRO Conf., Hobart 19 to 24 Feb. CRC for Temperate Hardwood Forestry, Hobart, Australia (1995). — BORRALHO, N. M. G.: Seed orchards or cuttings: which is the best? In: Proc. IUFRO Conf. on Silviculture and Improvement of Eucalypts. Pg. 330–336. Salvador, Brazil, 24 to 29 August. EMBRAPA (1997). — BORRALHO, N. M. G. and WILSON, P. J.: Inheritance of initial survival and rooting ability in *Eucalyptus globulus* LABILL. stem cuttings. Silvae Genetica **43**: 238–242 (1994). — COLLET, D.: Modelling Binary Data. Chapman & Hall, London (1991). — DE LITTLE, D. W. and RAVENWOOD, I. C.: Macropropagation of *E. nitens, E. globulus* and their hybrids. In: Proc., 11th RWG1 (Forest Genetics) Meeting. Coonawarra, S.A., Australia 114 (1991). — DUTKOWSKI, G. and POTTS, B.M.: Geographical patterns of genetic variation in *Eucalyptus globulus* ssp. *globulus* and a revised racial classification. Aust. J. Bot. **47**(1999). — ENGLAND, N. and BORRALHO, N. M. G.: Heritability of rooting success in *Eucalyptus globulus* stem cuttings. In: POTTS, B. M. et al. (Eds.): Eucalypt Plantations: Improving Fibre Yield and Quality. pp. 237-240. Proc. CRC-IUFRO Conf., Hobart 19 to 24 Feb. CRC for Temperate Hardwood Forestry, Hobart, Australia (1995). — GILMOUR, A.R., THOMPSON, R. and CULLIS, B. R.: Average information REML, an efficient algorithm for variance parameter estimation in linear mixed models. Biometrics **51**: 1440–1450 (1995). — HAINES, R. J. and WOOLASTON, R. R.: The influence of reproductive traits on the capture of genetic gain. Can. J. For. Res. **21**: 272–275 (1991). — HARTNEY, V. J.: Vegetative propagation of eucalypts *in vitro*. In: Mass Production Technology for Genetically Improved Fast Growing Forest Tree Species. Pp. 175–180. Proc. AFOCEL-IUFRO conference, Bordeaux 14 to 18 September 1992. AFOCEL, Nangis (1991). — LEAL, A. M. C. and COTTERILL, P. P.: Mass control-pollination of *Eucalyptus globulus.* In: Proc. IUFRO Conf. on Silviculture and Improvement of Eucalypts. Pp. 256–258. Salvador, Brazil, 24 to 27 August, EMBRAPA (1997). — LEMOS, L., CARVALHO, J., SOUSA, R. and BORRALHO, N. M. G.: Importance of additive and specific combining ability effects for rooting ability of stem cuttings in *Eucalyptus globulus.* Silvae Genetica **47**: 307–308 (1998). — LEROUX, J. J. and VAN STADEN, J.: Micropropagation and tissue culture of *Eucalyptus* – a review. Tree Physiology **9**: 435–477 (1991). — MACRAE, S. and COTTERILL, P. P.: Macropropagation and micropropagation of *Eucalyptus globulus:* means of capturing genetic gain. In: Proc. IUFRO Conf. on Silviculture and Improvement of Eucalypts. Pp. 102–110. Salvador, Brazil, 24 to 27 August, EMBRAPA (1997). — MURASHIGE, T. and SKOOG, F.: A revised medium for rapid growth and bioassay with tabacco tissue culture. Physiol. Plant. **15**: 473–497 (1962). — POTTS, B. M., VOLKER, P. W., HODGE, G. R., BORRALHO, N. M. G., HARDNER, C. M. and OWEN, J. N.: Genetic limitations in the exploitation of base populations of *Eucalyptus globulus* ssp. *globulus.* In: POTTS, B. M. *et al.* (Eds.): Eucalypt Plantations: Improving Fibre Yield and Quality. pp. 217–221. Proc. CRC-IUFRO Conf., Hobart 19 to 24 Feb. CRC for Temperate Hardwood Forestry, Hobart, Australia (1995). — RASMUSSEN, G. F., BOWER, D. L. M. and BORRALHO, N. M. G.: Genetic control of micro propagation characteristics in *Eucalyptus nitens* x *E. globulus* and implications for selection of clones. In: POTTS, B. M. *et al.* (Eds.): Eucalypt Plantations: Improving Fibre Yield and Quality. pp. 289–292. Proc. CRC-IUFRO Conf., Hobart 19 to 24 Feb. CRC for Temperate Hardwood Forestry, Hobart, Australia (1995). — SHAW, R. G.: The comparison of quantitative genetic parameters between populations. Evolution **45**: 143–151 (1991). — TIBBITS, W. N., WHITE, T. L., HODGE, G. R. and JOYCE, K. R.: Genetic control of rooting of stem cuttings in *Eucalyptus nitens.* Aust. J. Bot. **45**: 203–210 (1997). — WILLYAMS, D., WHITEMAN, P., CAMERON, J. and CHANDLER, S. F.: Inter- and intra-family variation in rooting capacity in micropropagated *Eucalyptus globulus* and *Eucalyptus nitens.* In: Mass Production Technology for Genetically Improved Fast Growing Forest Tree Species (AFOCEL-IUFRO Symposium 1992). Vol. II, pp. 177–181. Association Forêt Cellulose, Nangis, France (1992). — WILSON, P. J.: The development of new clones of *Eucalyptus globulus* and *E. globulus* hybrids by stem cuttings propagation. In: Mass Production Technology for Genetically Improved Fast Growing Forest Tree Species (AFOCEL-IUFRO Symposium 1992). Vol. I, pp. 379–386. Association Forêt Cellulose, Nangis, France (1992). — WILSON, P. J.: Propagation characteristics of *Eucalyptus globulus* LABILL. ssp. *globulus* stem cuttings in relation to their original position in the parent shoot. J. Hort. Sci. **68**: 715–724 (1993). — ZOBEL, B. J.: Clonal forestry in the Eucalyptus. In: AHUJA, M. R. and LIBBY, W. J. (Eds.): Clonal Forestry II, Conservation and Application. pp. 140–148. Springer-Verlag, Berlin, Heidelberg (1993).

Heartwood and Sapwood Variation in Mature Provenance Trials of *Pinus sylvestris*

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(Received 9th September 1998)

Abstract

In two Swedish provenance test series of Scots pine *(Pinus sylvestris* L.) from 1911 and 1955, only limited provenance differences were found in number of heartwood and sapwood annual rings and in the width of heartwood and sapwood. Variance components were smaller than for tree size traits. Correlations between heartwood and sapwood annual rings were negative and significant, while weak correlations were indicated between heartwood and sapwood width. Correlations with tree size traits were weaker in the young test series than in the older series. The results indicate that stand characteristics (such as stand density) have stronger influence than